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TITLE: Nematode-extracted anticoagulant protein

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INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---|---|---|---|---|
| Vlasuk; George Phillip | Carlsbad | CA | N/A | N/A |
| Stanssens; Patrick Eric Hugo | St-Martens-Latem | N/A | N/A | BEX |
| Messens; Joris Hilda Lieven | Antwerp | N/A | N/A | BEX |
| Lauwereys; Marc Jozef | Haaltert | N/A | N/A | BEX |
| Laroche; Yves Rene | Brussels | N/A | N/A | BEX |
| Jespers; Laurent Stephane | Tervuren | N/A | N/A | BEX |
| Gansemans; Yannick Georges Jozef | Bredene | N/A | N/A | BEX |
| Moyle; Matthew | Escondido | CA | N/A | N/A |
| Bergum; Peter W. | San Diego | CA | N/A | N/A |

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CLAIMS:

We claim:
1. An isolated protein having Factor Xa inhibitory activity and having one or more NAP domains, wherein each NAP domain includes the sequence:
Cys-A1-Cys-A2-Cys-A3-Cys-A4-Cys-A5-Cys-A6-Cys-A7-Cys-A8-Cys-A 9-Cys-A10, wherein
(a) A1 is an amino acid sequence of 7 to 8 amino acid residues;
(b) A2 is an amino acid sequence;
(c) A3 is an amino acid sequence of 3 amino acid residues;
(d) A4 is an amino acid sequence;
(e) A5 is an amino acid sequence of 3 to 4 amino acid residues;
(f) A6 is an amino acid sequence;
(g) A7 is an amino acid residue;
(h) A8 is an amino acid sequence of 11 to 12 amino acid residues;
(i) A9 is an amino acid sequence of 5 to 7 amino acid residues; and
(j) A10 is an amino acid sequence;
wherein each of A2, A4, A6 and A10 has an independently selected number of independently selected amino acid residues and each sequence is selected such that each NAP domain has in total less than about 120 amino acid residues and wherein said isolated protein is derived from a hematophagous nematode species.
2. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues.
3. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a is selected from the group consisting of Ala, Arg, Pro, Lys, Ile, His, Leu, and Thr, and A3.sub.b is selected from the group consisting of Lys, Thr, and Arg.
4. The protein of claim 3, wherein A3 is selected from the group consisting of
Glu-Ala-Lys,
Glu-Arg-Lys,
Glu-Pro-Lys,
Glu-Lys-Lys,
Glu-Ile-Thr,

Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys.
5. The protein of claim 1, wherein A4 is an amino acid sequence having a net anionic charge.
6. The protein of claim 1, wherein A7 is Val.
7. The protein of claim 1, wherein A7 is Ile.
8. The protein of claim 1, wherein A8 includes the amino acid sequence -A8.sub.a -A8.sub.b -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - [SEQ. ID. NO. 68], wherein
(a) A8.sub.a is the first amino acid residue in A8,
(b) at least one of A8.sub.a and A8.sub.b is selected from the group consisting of Glu or Asp, and
(c) A8.sub.c through A8.sub.g are independently selected amino acid residues.
9. The protein of claim 8, wherein
(a) A8.sub.a is Glu or Asp,
(b) A8.sub.b is an independently selected amino acid residue,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
10. The protein of claim 9, wherein -A8.sub.c-A 8.sub.d-A 8.sub.e -A8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
11. The protein of claim 8, wherein
(a) A8.sub.a is an independently selected amino acid residue,
(b) A8.sub.b is Glu or Asp,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
12. The protein of claim 11, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A 8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
13. The protein of claim 8, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
14. The protein of claim 1, wherein A10 includes an amino acid sequence selected from the group consisting of
Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and
Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].
15. The protein of claim 14, wherein A10 includes the amino acid sequence
Glu-Ile-Ile-His-Val [SEQ. ID. 74].
16. The protein of claim 15 having a NAP domain with an amino acid sequence substantially the same as that of AcaNAP5 [SEQ. ID. NO. 40] or AcaNAP6 [SEQ. ID. NO. 41].
17. The protein of claim 14, wherein A10 includes the amino acid sequence
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75].
18. The protein of claim 14, wherein A10 includes the amino acid sequence
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76].
19. The protein of claim 14, wherein A10 includes the amino acid sequence
Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].
20. The protein of claim 1, wherein said nematode species is selected from the group

consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
21. The protein of claim 1, wherein
(a) A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues;
(b) A4 is an amino acid sequence having a net anionic charge;
(c) A7 is selected from the group consisting of Val and Ile;
(d) A8 includes an amino acid sequence selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73]; and
(e) A10 includes an amino sequence selected from the group consisting of
Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and
Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].
22. The protein of claim 21 having a NAP domain substantially the same as NAP domains selected from AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
23. The protein of claim 22, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
24. The protein of claim 1, wherein
(a) A3 is selected from the group consisting of
Glu-Ala-Lys,
Glu-Arg-Lys,
Glu-Pro-Lys,
Glu-Lys-Lys,
Glu-Ile-Thr,
Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys;
(b) A4 is an amino acid sequence having a net anionic charge;
(c) A7 is Val or Ile;
(d) A8 includes an amino acid sequence selected from the group consisting of
A3.sub.a -A3.sub.b -Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 78],
A3.sub.a -A3.sub.b -Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 79],
A3.sub.a -A3.sub.b -Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 80],
A3.sub.a -A3.sub.b -Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 81], and
A3.sub.a -A3.sub.b -Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 82], wherein at least one of A3.sub.a and A3.sub.b is Glu or Asp;
(e) A9 is an amino acid sequence of five amino acid residues; and
(f) A10 includes an amino acid sequence selected from the group consisting of
Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and
Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].
25. The protein of claim 24 having a NAP domain substantially the same as NAP domains selected from AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
26. The protein of claim 24, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
27. A pharmaceutical composition comprising the protein of claim 1.
28. A pharmaceutical composition comprising the protein of claim 21.
29. A pharmaceutical composition comprising the protein of claim 24.
30. A method of inhibiting blood coagulation comprising administering a protein of claim 1 with a pharmaceutically acceptable carrier.
31. A method of inhibiting blood coagulation comprising administering a protein of claim 21 with a pharmaceutically acceptable carrier.
32. A method of inhibiting blood coagulation comprising administering a protein of claim 24 with a pharmaceutically acceptable carrier.
33. A protein of claim 1, wherein said protein has two NAP domains.
34. A protein of claim 21, wherein said protein has two NAP domains.
35. A protein of claim 24, wherein said protein has two NAP domains.
36. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 1.
37. A method according to claim 36 wherein said pathologic condition is disseminated

intravascular coagulopathy.
38. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 21.
39. A method according to claim 38 wherein said pathologic condition is disseminated intravascular coagulopathy.
40. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 24.
41. A method according to claim 40 wherein said pathologic condition is disseminated intravascular coagulopathy.
42. An isolated protein having Factor Xa inhibitory activity selected from the group consisting of AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
43. A pharmaceutical composition comprising a protein selected from the group consisting of AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
44. A method of inhibiting blood coagulation comprising administering a protein selected from the group consisting of AcaNAP5 [SEQ. ID. NO. 40] and AcaNAP6 [SEQ. ID. NO. 41].
45. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 42.
46. A method according to claim 45 wherein said pathologic condition is disseminated intravascular coagulopathy.